THE DEVELOPMENT OF THE ADRENAL GLANDS OF BIRDS

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VICTOR J. HAYS

A THESIS

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THE DEVELOPMENT OF THE ADRENAL GLANDS OF BIRDS

VICTOR J. HAYS

From the Laboratories of Animal Biology of the State University of Iowa

EIGHT FIGURES

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INTRODUCTION

Although the development of the adrenal glands has been studied for the various classes of vertebrates for a number of years and by many investigators of recognized ability, there seems to be no very general agreement in their conclusions; and several opposing theories have been developed as a result. This is especially true of the observations on the development of the adrenals of birds. Here the field is in a most chaotic condition and a review of the literature shows, that while one theory may have the weight of evidence in its favor, each of them is supported by a number of investigators whose ability is of the highest order. No minute description of the development of the vascular system of the adrenal glands of birds has as yet appeared. The development of the vascular system of the adrenal glands of mammals has been reported; but this cannot be taken as a

criterion for the development in birds, since in birds there can be no sharp division of the glands into cortex and medulla.

The object of this investigation is to determine the source and manner of development of the various systems of the adrenal glands of birds, and to make clear the relationship existing between these different systems in the birds.

It is safe to say that there is no longer any doubt as to the nature of the adrenal glands, since the fact is well established that in the higher vertebrates they represent the more or less complete union of the interrenals and suprarenals of the lower vertebrates. The adrenal glands of the higher vertebrates are then a pair of organs, each representing an interrenal and a suprarenal gland of the lower classes of vertebrates. In the adrenals of mammals, the cortical substance represents the interrenals, while the medullary substance corresponds to the suprarenal glands of the lower vertebrates. Since in the case of birds there is no true medulla, the term 'chromaffin substance' will be used in place of the term 'medullary substance.'

According to the different investigators, the cortical substance has been derived from several possible sources; the mesenchyme, the mesonephros, the germinal epithelium, the peritoneal epethelium, and the sympathetic ganglia.

Gottschau ('83) and Minot ('97) took the view that the cortical substance develops from the mesenchyme, the former working with mammalian embryos and the latter with human embryos. The theory of mesonephric origin was supported by Semon ('87) and C. K. Hoffmann ('92), both working with the embryos of birds. Von Mihalcovics ('85) working with reptiles, and Janošik ('83, '90), Fusari ('93), and Loisel ('04), working with bird embryos, found a very intimate relationship between the adrenals and the genital glands and took the view that the adrenals develop from the germinal epithelium, so far as the cortical substance is concerned. O. Schultze ('97), from his obvserations made on embryos of Vespertillio murinus, concluded that the cortical substance of the adrenal gland arises from the sympathetic ganglia. The following observers support the theory that the cortical substance of the adrenal glands develops from the

peritoneal epithelium. Valenti ('93) and Souli ('03), working with bird embryos, and Kuntz ('12), working with embryos of Thalassochelys caretta. This view is also supported by Poll ('06). The above citations do not cover the entire field but are given only to show the various theories which have been proposed to account for the cortical substance of the adrenal glands.

Several theories have also been proposed to account for the development of the chromaffin substance of the glands. Here again there is a lack of agreement in the conclusions of the various investigators, as was the case, in the observations on the development of the cortical substance. Gottschau ('83) and Minot ('97) derived the chromaffin substance from the mesenchyme, the former from observations made on mammalian embryos and the latter, from human embryos. Von Mihalcovics ('85), from observations made on reptilian embryos, came to the conclusion that the chromaffin substance of the glands is derived from the germinal epithelium. This theory was upheld by Janosik ('83, '90) and Valenti ('89, '93), both working with embryos of birds. Leydig ('53) described the interrenals and suprarenals of fishes and came to the conclusion that the suprarenals are derived from the sympathetic nervous system. Balfour ('78) in his classical work on the elasmobranch fishes, shows conclusively that the suprarenals are derived from the sympathetic ganglia along the abdominal aorta. Since that time many investigations have verified these conclusions and it is hard to account for the fact that many of the earlier investigators refused to accept the results of the work of Leydig and Balfour. Among the later investigators to hold the theory of sympathetic origin of the chromaffin substance are: Fusari ('90, '93), H. Rabl ('91), Minervini ('04), and Loisel ('04). These investigators all worked with bird embryos. Souli ('03) and C. K. Hoffmann, (89, 92), working with the embryos of birds and reptiles, came to the theory of sympathetic origin. This theory was also supported by the work of Poll ('06) in which he used the embryos of mammals, reptiles, and birds. Kuntz ('12), from observations made on the embryos of Thalassochelys caretta, concludes that the chromaffin substance develops from the analgen of the

prevertebral sympathetic plexuses. The above citations, while they do not cover the entire field, serve to show the confusion which exists concerning the development of the adrenal glands. A complete bibliography will be found in the work of Poll ('06).

There are two general theories to account for the origin of the cortical and chromaffin substances of the adrenal glands; the theory of homogeneous origin and that of heterogeneous origin. The supporters of the theory of homogeneous origin have in turn derived the adrenal glands from the sympathetic nervous system, from the mesenchyme, and from the germinal epithelium. There is the same lack of agreement among the supporters of the theory of heterogeneous origin. These investigators have in turn derived the glands from the mesonephros and the peripheral part of the sympathetic nervous system, the germinal epithelium and the sympathetic nervous system, and from the peritoneal epithelium and the sympathetic nervous system. Poll, from extensive observations and from a thorough review of the literature, shows that the weight of evidence favors the theory that the cortical substance of the adrenal glands of all vertebrates is derived from the peritoneal epithelium and that the chromaffin substance develops from the cells which break away from the anlagen of the peripheral part of the sympathetic nervous system.

Very little work has been done on the development of the blood vessels of the adrenal glands. Flint ('00) has worked out the blood vessels of the adrenals of mammals and reports a very interesting condition existing in this class of animals, especially as regards the venous circulation. According to this investigator the venous system may be compared to a tree, the terminal twigs uniting to form larger branches and as a natural result of this process a large central vein is formed. He found that in most cases this central vein opens into the postcava as a single vein. In the dog, however, the central veins of the posterior and anterior lobes of the gland do not unite, but open into the postcava separately. This description refers only to the venous system of the medullary part of the gland. The venous system of the cortical part of the gland is of no great importance, being

composed of the terminal twigs of the medullary venous tree. The arteries of the gland, according to Flint, are derived from five sources: A. phrenica, A. phrenica accessorius, A. lumbalis, A. renalis, and the abdominal aorta. These arteries branch out on the capsule of the gland forming a network of blood vessels over the entire gland. These branches finally enter the cortex at various points and break up into capillaries, the terminal branches penetrating the medulla for a short distance.

Miller ('03), working on the development of the postcaval vein in birds, did not make any attempt to work out the development of the veins in the adrenal glands other than to determine their origin. He concluded that the veins of the left adrenal develop from the subcardinal vein and probably those of the right gland are of the same origin.

Minot ('00) distinguishes between capillaries and the venous blood vessels found in several organs of the vertebrates, among these being the adrenal gland. He finds these blood vessels differing from capillaries in size, shape, relation to other tissues, and in their method of development. According to this author, these blood vessels which he calls sinusoids are larger than capillaries and are irregular in section. The walls of sinusoids are composed of a single layer of endothelial cells resting upon the parenchyma of the organ, while a capillary always has a connective tissue wall upon which the endothelial layer rests. The manner of development also differs, the capillaries developing from a chain of vasoformative cells which becomes hollowed out and connected with a vessel already formed, while sinusoids develop by the outpushing of the endothelium of the wall of a pre-existing blood vessel.

The vascular spaces observed and described by Kuntz ('12) in the adrenals of Thalassochyles caretta are undoubtedly identical with the sinusoids of Minot. Flint ('00) finds no sinusoids in the adrenals of mammals and is of the opinion that the investigators who have reported them used sections which were too thin to show the true structure of the walls of the blood vessels.

The following observations are based exclusively on embryos and adult of the domestic fowl (Gallus domesticus). All speci-

mens were fixed with chrom-aceto-formaldehyde and stained by the iron hematoxylin method. Embryos were injected with india ink after the method of Knower ('08). The adults were injected with a gelatin mass. Sections used for the study of the development of the vascular system were cut to a thickness of 20 micra. All other sections were 10 micra thick.

It gives me great pleasure to express my indebtedness to Prof. F. A. Stromsten for many helpful suggestions during my investigation of this subject and also for reading the manuscript. I take the greatest pleasure in acknowledging my indebtedness to Prof. G. L. Houser for suggesting the subject of this investigation and for many helpful suggestions during its progress.

OBSERVATIONS

The early development of the cortical substance

The cells which are later to form the cortical substance of the adrenal glands of birds are first seen in the 96th hour of incubation. They appear as a thickening of the peritoneal epithelium, ventral and mesial to the mesonephros, ventral to the abdominal aorta, and dorsal to the hind gut which is open at this time (fig. 1, *ad.*). The developing cells push in dorsally from the epithelium upon which they rest and become larger and more nearly circular in outline than those cells from which they arose, that is, the cells of the peritoneal epithelium. The nuclei are correspondingly enlarged and mitotic figures may be seen in nearly all of them. The nuclei also differ from those of the parent cells in their staining properties, these nuclei all being less deeply stained and less granular than those of the peritoneal epithelium. It is probably due to the fact that the anlagen of the cortical substance appear so early in the development of the chick, that earlier investigators, using embryos which had passed this stage of development, derived the cortical substance from other sources.

During this early period of incubation the development of the cortical substance goes on with astonishing rapidity, and nine hours later, during the 105th hour of incubation, the cortical

cells have piled up on the peritoneal epithelium so that a solid body is formed on each side of the base of the mesentery. In the meantime, folds have appeared in the peritoneal epithelium which throw these cell groups further from the base of the mesentery,

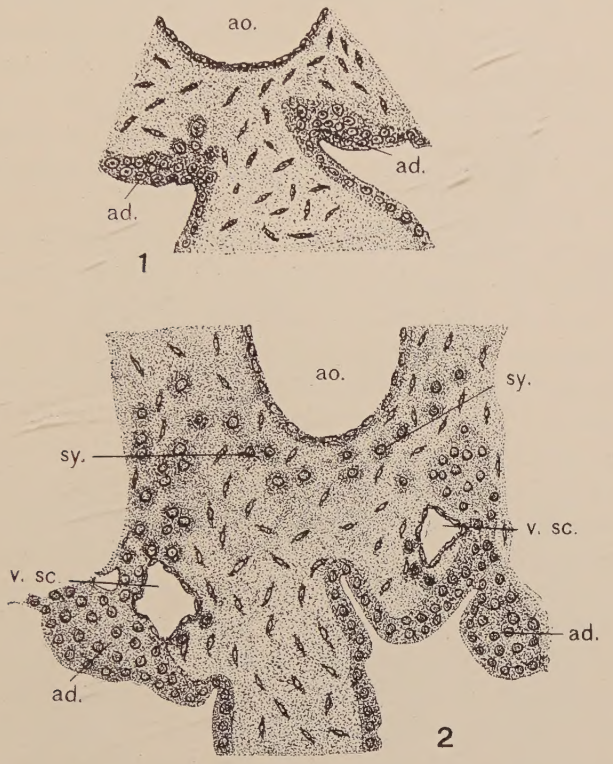


Fig. 1 Transverse section through the adrenal region of a 96-hour chick embryo; *ad.*, anlagen of the cortical substance; *ao.*, aorta. × 130.

Fig. 2 Transverse section through the adrenal region of a 105-hour chick embryo; *ad.*, anlagen of the cortical substance; *ao.*, aorta; *sy.*, anlagen of the prevertebral sympathetic plexuses; *v. sc.*, subcardinal vein. × 130.

laterally. At this period they lie just mesial to the ventral side of the mesonephros. This is possible because the mesonephros lies closer to the median line than in the preceding stage, due chiefly to the fact that it has been growing rapidly during this period. The character of the cortical cells has not changed

during this period, but their migration has gone on rapidly until a chain of cells can be traced from the group resting on the peritoneal epithelium, to a point just slightly dorsal to the ventral level of the aorta (fig. 2, *ad.*). These cells then lie between the aorta and the mesonephros, in the mesenchyme. In this migration most of the cells pass laterally to the subcardinal veins but this does not hold true for all of them, since a few of them take a path median to these veins. The shape of these cells and their nuclei, together with their staining properties, make them easily identified and the course of their migration can be followed without difficulty.

During the next fifteen hours, or after 120 hours of incubation, the cortical cells have become detached from the peritoneal epithelium and all of them have migrated dorsally. At this stage of development they appear as scattered cell groups reaching from the dorsal level of the subcardinal veins to the middle level of the aorta. They have reached about the same level on both the right and left sides of the aorta, though those on the right side may be slightly in advance of those on the left. These cell groups are scattered through the mesenchyme between the mesonephros and the aorta and have practically invaded the entire region. The nuclei are still circular in section and show well developed mitotic figures. Occasionally cells may be seen undergoing division. Isolated cells are still circular in outline but those which are found in groups have become more or less flattened by contact with the other cells of the group and present an oval outline. They may still be identified from anything which has yet appeared by their nuclei and staining properties (fig. 3, *ad.*). The relative position of the cortical cells at this period is shown by figure 6. It is seen that they lie on the dorsal side of the subcardinal veins, lateral and ventral to the aorta, and mesial and ventral to the postcardinal veins. The region which they occupy extends posteriorly to a point about level with the anastomosis of the subcardinal veins in the median line, ventral to the dorsal aorta.

From the 120th to the 130th hours of incubation there is a great increase in the mass of the cortical substance. This is

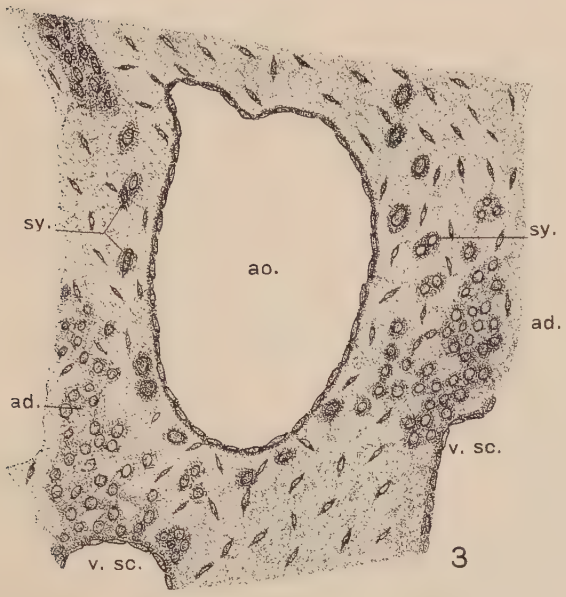


Fig. 3 Transverse section through the adrenal region of a 120-hour chick embryo; *ad.*, anlagen of the cortical substance; *ao.*, aorta; *sy.*, anlagen of the prevertebral sympathetic plexuses; *v. sc.*, subcardinal vein. × 90.

Fig. 4 Transverse section through the adrenal region of a 130-hour chick embryo; *ad.*, anlagen of the cortical substance; *ao.*, aorta; *ch. a.*, anlagen of the chromaffin substance; *mes.*, mesonephros; *sy.*, anlagen of the prevertebral sympathetic plexuses; *v. sc.*, subcardinal vein. × 90.

due, partly to the fact that the cells are no longer scattered through the mesenchyme, but have collected in large groups, and partly to the fact that the number of these cells has been increased by the division of the older cells present in this region. At this stage the cells are arranged in large solid groups lying dorso-mesial to the subcardinal veins and ventral to the mesonephric arteries which run over the anterior ends of these cell groups. These cells then occupy the region between the aorta and the mesonephros in the region outlined above. Owing to the close arrangement of the cells, they are losing their regular shape, but the nuclei remain circular in outline and continued development is shown by the presence of mitotic figures (fig. 4, *ad.*).

After 144 hours incubation the cells have become more closely grouped than in the preceding stages and are found in large oval masses on each side of the aorta. The nuclei have become more granular but still contain mitotic figures. The cells are becoming more irregular in outline and, on account of the proximity of these cells to the mesonephros, and on account of the close resemblance between them at this time, it is difficult to distinguish one from the other. Such conditions, doubtless, are responsible for the conclusions of some of the earlier investigators that the cortical substance of the adrenals is derived from the mesonephros. Careful investigation reveals a thin layer of flattened mesenchyme cells between these two bodies.

Twenty-four hours later, during the 168th hour of incubation, the mass of the cortical substance has greatly increased. The cells have arranged themselves in irregular chains and have taken a roughly hexagonal shape. The nuclei stain much darker than previously but they still show mitotic figures in great numbers, showing that the cortical substance of the gland is still increasing by division of its own cells. The mass of cortical substance is roughly circular in section at this time and occupies practically the same level as the aorta and has about the same cross sectional area through the center. The mesonephros has developed ventrally until it is in contact with the adrenal only at its dorso-mesial angle. The subcardinal veins still lie on the ventral

border of the glands. At this period of development, connective tissue fibers are collecting around the gland, giving promise of a connective tissue capsule later. A few of the fibers are seen within the body of the gland, between the cords of cells.

The cortical substance continues to grow rapidly during the next twenty-four hours and after 192 hours of incubation its cross sectional area is fully twice as great through the center as that of the aorta. The gland is about 2 mm. long at this period. The cell mass is becoming less dense than it has been for some time. A large number of the nuclei still show mitotic figures but in many of the cells these figures are no longer present. At this time, blood cells may be seen in the relatively large openings between the cords of cortical cells.

Little change in the form and size of the gland is seen during the next twenty-four hours. The greatest changes are seen in the internal arrangement of the cells. The gland has become much more vascular during this period and many more spaces have appeared between the cords. The cell cords have become very dense and compact, making it difficult to see the outline of the individual cell.

The above observations lead to but one conclusion, namely, that the anlagen of the cortical substance of the adrenal glands arise as groups of cells which proliferate from the peritoneal epithelium.

Early development of the chromaffin substance

The observations on the development of the adrenal gland show that the anlagen of the cortical substance arise from the peritoneal epithelium. Observations on the origin of the chromaffin substance seem to show that it arises, not from the same source as the cortical substance, but from the anlagen of the prevertebral sympathetic plexuses. It is evident then that the adrenal glands arise from two separate germ layers, namely, the mesoderm and the ectoderm.

After 120 hours of incubation, large oval cells are seen migrating ventrally from the sympathetic trunks on each side of the aorta. These cells migrate singly in most cases and most of

them pass around to the ventral side of the aorta and later form the prevertebral sympathetic plexuses (fig. 3, *sy.*). At this stage of development the anlagen of the cortical substance are a loose group of cells on each side of the aorta. The cells of sympathetic origin migrate in a path which causes them to pass between the aorta and the groups of cortical cells. At this time there is no connection between these two kinds of cells. The two kinds of cells, cortical and sympathetic, are easily distinguished from one another by their size and affinity for stains, the latter being the larger and taking the deeper stain.

The first evidence of any connection between the anlagen of the prevertebral sympathetic plexuses and the chromaffin substance is seen after 130 hours of incubation. The cortical cells have arranged themselves in large, compact masses by this time and have taken a definite outline. At this time, some of the cells migrating from the sympathetic trunks turn off ventrally in the region of the adrenals and either enter them, or become attached to the surface of the cell groups. Figure 4 (*sy.*) shows several of these cells on the inner edges of the groups of cortical cells, and on the right, one cell may be seen which has penetrated to the center of the cortical substance. This development continues for some time and these new elements, the cells of sympathetic origin, do not seem to differ in any way from those which pass on to form the prevertebral sympathetic plexuses. These cells, then, are indifferent in nature. As the growth of the embryo goes on, more of these cells are found entering the cortical substance of the gland and collecting, in most cases, in groups of two or three. Single cells, however, are found scattered throughout the cortical substance. During this period they may be found almost anywhere within the cortical substance and a great many are found around the surface of the glands.

After 168 hours of incubation, the cells which are to form the chromaffin part of the gland are beginning to show some differentiation. Those which have entered the cortical substance are no longer large circular cells with round, clear nuclei. The shape is becoming irregular, as a general rule, and the cells are smaller than originally. The nuclei are oval and have become quite

granular, in many cases, even more so than those of the cortical cells. They are most easily distinguished from the latter cells by means of their staining properties, both nucleus and cytoplasm taking a deeper blue color with the iron hematoxylin method. These invading cells, at this stage of development, show a tendency to arrange themselves in cords throughout the cortical substance, though many solitary cells are also found. This seems to be the height of the migration of the cells from the sympathetic trunks and at this time the mesenchyme around the glands is shot full of them, and they can be seen entering the glands from all sides.

During the next twenty-four hours, the chromaffin cells within the glands have increased greatly in number and most of the sympathetic cells have disappeared from the mesenchyme around the glands. At this time the chromaffin cells are arranged in cords, many of which have pushed in close to the venous blood vessels. This location with regard to the venous circulation cannot be taken as a general rule at this period of development, since a great number of these cords do not seem to bear any relation to the blood vessels.

The arrangement of the chromaffin cells undergoes a marked change during the next twenty-four hours, 216 hours' incubation. The cells were first found scattered, either singly, or in small groups, throughout the cortical substance. Later they became arranged in cords or columns. At this period the cells cords break down, but do not return to the original condition of solitary cells scattered throughout the cortical substance. Instead of this scattered arrangement, the cells are found in small groups arranged around the venous blood vessels. Of course, not all of the groups are so situated, since the cords from which they originated were not all in contact with the blood vessels.

These observations bear out the contention that the chromaffin substance of the glands does not arise from the mesenchyme or germinal epithelium, but from the anlagen of the prevertebral sympathetic plexuses. These cells enter the cortical substance as indifferent cells and later become differentiated to form the chromaffin substance of the glands.

Development after 216 hours' incubation

After 216 hours' incubation the characteristic features of the adrenal glands are firmly established and the development of the glands from that time up to hatching is chiefly one of growth so far as the cortical and chromaffin cells are concerned. The glands increase in volume slowly and become more vascular until, at the end of the period of incubation, they have the

Fig. 5 Transverse section through the adrenal gland of a 264-hour chick embryo; *ao.*, aorta; *ch.*, chromaffin substance; *co.*, cortical substance; *si.*, sinusoids; *sy.*, anlagen of the prevertebral sympathetic plexuses. × 90.

appearance, in section, of a large number of groups of cells almost surrounded by blood vessels.

An idea of the structure of the gland may be had by referring to figure 5. Here the gland is seen lying between the kidney and the aorta, practically filling this region. The substance of the gland is cut up irregularly by venous sinusoids which form a network throughout the entire gland. The cortical cells are arranged in irregular columns which pass around these blood vessels and seem to form the foundation for all other elements

of the gland. The chromaffin cells have no regular arrangement, but are found in groups varying from two or three, up to thirty or forty cells each. The only regularity to be seen in the chromaffin groups is in their relation to the venous blood vessels. Except in exceptional cases, at least a part of each group is in direct contact with at least one of these blood vessels.

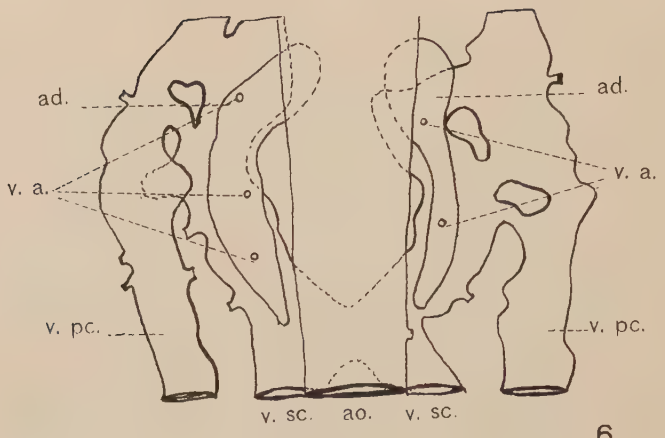
The connective tissue, which was first seen forming around the glands at the 168th hour of incubation, develops very slowly, but after about seventeen days of incubation a dense capsule has been formed around each gland. The connective tissue is confined almost entirely to the surface of the gland but in several places rather large masses of it may be seen entering the substance of the gland. This connective tissue breaks up at once and within the gland only very small fibers are found. These fibers are found only between the cords of cortical cells.

The development of the venous system

Owing to the structure of the adrenal glands of birds, the development of the blood vessels cannot be taken up separately for the cortical and chromaffin parts, as has been done for mammals.

The blood vessels of the adrenal gland develop so slowly that for specimens taken twelve hours apart, very little difference can be seen. For this reason it is very difficult to determine at exactly what age they first appear. The process is a gradual one and each condition blends perfectly into those immediately preceding, and those immediately following it.

As early as the 120-hour stage of development a few scattered blood cells are found throughout the anlagen of the glands, but no more are found than are present in the surrounding mesenchyme tissue. No direct connection with any blood vessels can be seen at this stage of development but in several places the wall of the subcardinal vein pushes out dorsally into the anlagen of the gland for a very short distance. No break or division of the wall of the vein can be seen at this time. Figure 6 shows the



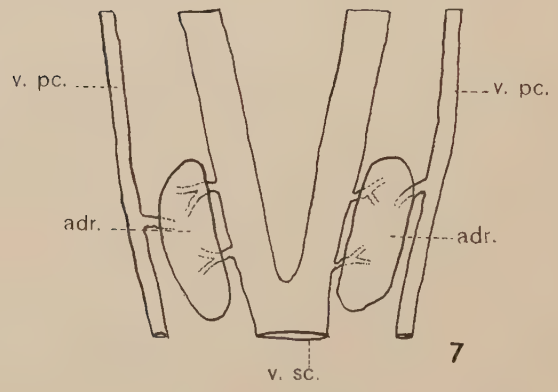


Fig. 6 Reconstruction of the vascular system in the adrenal region of a 120-hour chick embryo; dorsal aspect; *ad.*, anlagen of the cortical substance; *ao.*, aorta; *v. a.*, adrenal vein; *v .pc.*, postcardinal vein; *v. sc.*, subcardinal vein.

Fig. 7 Semi-diagrammatic drawing of the connection between the subcardinal and the postcardinal veins through the adrenal in a 168–hour chick embryo; ventral aspect; *adr.*, adrenal gland; *v. pc.*, postcardinal vein; *v. sc.*, subcardinal vein.

relation of the subcardinal veins to the glands and in several places the outpushing of the veins into the glands may be seen.

Sections of the glands at the 130-hour stage of development show that the subcardinal veins have pushed further into the

gland tissue than in the previous stage. No great modification of the gland can be seen and there is no apparent increase in the number of blood cells found in the gland. The evaginations of the subcardinal veins are very small at this time, but can be traced by the structure of their walls, which at this time are composed of only a single layer of endothelial cells. Their walls are of the same structure as those of the subcardinal veins at this period of development.

This development continues during the following fourteen hours so that at this period, 144 hours' incubation, these newly formed blood vessels have reached almost to the dorsal side of the gland. In several places, lateral branches are forming but these extend for only a very short distance. In no case was less than two of these venous trees found and in most cases three or four of them were present. This means that the venous blood vessels of the gland are formed, not by the division of one larger vein, but from several which push in from the subcardinal veins.

The development of the venous system continues by the branching of the vessels present in the gland until, after 168 hours of incubation, the gland has the appearance in section of having many irregular pieces cut out of its interior. At this period of development a new venous connection appears in the glands of birds (fig. 7). By the growth of the glands they come to lie ventro-median to the postcardinal veins. At this time, these veins turn ventrally to form the renal portal system, and in the region of the adrenals a branch is given off to the glands. Each postcardinal gives off a branch to the gland on its side of the body cavity. These veins push into the glands but instead of branching after the manner of the subcardinals and forming a system within the glands, they open directly into the blood vessels already present in the glands. In other words, they open into the venous tree already formed from the subcardinal veins. This condition naturally leads to the conclusion that there is a portal system formed in the adrenal glands of birds which might be called the adrenal portal system.

At this time the aorta and the postcardinal and subcardinal veins show connective tissue in their walls, upon which the

endothelial lining rests. This is not true of the venous blood vessels in the glands. These show no connective tissue in their walls and are larger than any capillaries found at this time. Owing to the difference in size and structure of these vessels I shall not call them capillaries, but shall adopt the term 'sinusoids,' proposed by Minot for this type of blood vessel.

The development of the sinusoids goes on steadily, but after 216 hours of incubation there does not appear to have been any appreciable increase in their number. The most noticeable change in the appearance of the gland is in the great increase in the size of the sinusoids. This growth, however, has in no way affected the nature of their walls, and they are still made up of a single layer of endothelial cells (fig. 5, *si.*). The adrenal portal system has broken down at this time. It is a transitory condition, persisting through the eighth and ninth days of incubation only. It is significant that this connection with the post-cardinal vein should disappear as soon as the sinusoids have increased greatly in size. It should also be remembered that the greatest activity in the development of the chromaffin substance took place during the existence of this system.

Until after 240 hours of incubation, the sinusoids are found to open into the subcardinal veins in many places and by means of no very definite vessels. At this stage, 240 hours, the sinusoids seem to join into two groups near the posterior end of each gland and enter the subcardinal veins by means of four well defined veins, two for each gland. There is no evidence of a central vein running longitudinally through the gland, but this is to be expected since the sinusoids do not arise from the branching of a single vein, but from four or five veins which push in from the subcardinal vein. Each one of these veins then forms a system of sinusoids by repeated branching in the gland. This later development is then the combination of several of these systems at their bases to form a larger system. Since there are two veins found opening into the subcardinal vein, it follows that these numerous systems have combined to form two distinct systems of sinusoids in each gland. These systems are distinct only in point of origin, since there are numerous anasto-

moses formed between the sinusoids of the different systems and between the sinusoids of the same system.

The later development of the venous system is in no way remarkable. The terminal sinusoids continue to branch slowly up to the end of the period of incubation. At this period the gland is filled with sinusoids, so much so that in section they appear to occupy nearly one-half of the entire volume of the gland.

There can be no doubt as to the origin of the venous system of the adrenal glands of birds. It develops from inpushings of the subcardinal veins and penetrates the glands by means of numerous branches which in almost every case are directly in contact with the chromaffin substance.

The development of the arterial system

Observations on the development of the arteries of the adrenal glands reveal no conditions which are in any way out of the ordinary, and the condition is the same as that of any organ of this type.

The earliest connection found between the glands and any of the nearby arteries appears after 120 hours of incubation. At this time a small blood vessel is seen passing into the cortical substance of each gland from the anterior pair of mesonephric arteries. At this period the walls of these vessels are not very distinct, and within the gland no capillaries can be seen. For some time very little progress can be seen in the development of the arteries. After 144 hours of incubation the only increase in the complexity of the arterial system is the appearance of a very few indistinct capillaries within the cortical substance.

During the next twenty-four hours the arterial system of the glands develops rapidly and several new vessels appear during this period. An artery is given off by each of the anterior mesonephric arteries just before they enter the mesonephric glands. These arteries run anteriorly, one along the lateral border of each adrenal gland and two branches are given off to each gland by its respective artery, one near the posterior and the other near the anterior end of the gland, and each sends

branches into the cortical part of the gland. This does not occur, however, until after several branches have been formed on the surface of the gland and instead of a large artery penetrating the gland, directly, it is broken up and the branches are very small when they enter the substance of the gland. Still another arterial connection appears at this time. This is a small artery which runs directly from the aorta to the posterior

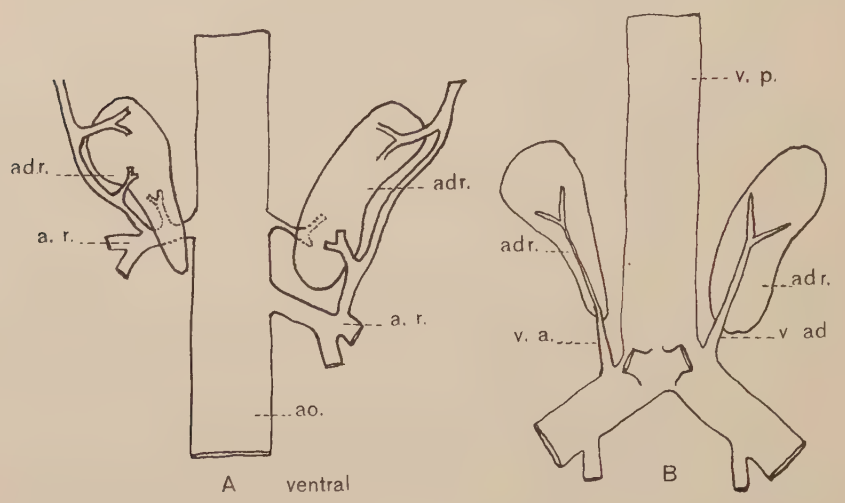


Fig. 8 A, The arteries of the adult fowl in the region of the adrenal glands; ventral aspect; *adr.*, adrenal gland; *ao.*, aorta; *a. r.*, renal artery. B, The veins of the adult fowl in the region of the adrenal gland; ventral aspect; *adr.*, adrenal gland; *v. a.*, adrenal vein; *v.p.*, postcava. × 2.

end of the left gland. This artery also branches on the surface of the gland before entering the cortical substance. Within the glands all these arteries divide still further and the terminal branches are so small that in section they appear to be smaller than the blood cells. It is probable that in the living gland these capillaries are somewhat larger than in the sections observed. None of the capillaries within the gland were found in the chromaffin substance but there can be no doubt that at least the terminal branches sometimes penetrate this part of the gland.

Here again, the greatest growth of the chromaffin substance is accompanied by a correspondingly rapid development of the vascular system.

This practically completes the development of the arterial system of the glands. At a later period, about 192 hours, the connection between the mesonephric artery and the left adrenal disappears. This artery which disappears is not the one which arises from the branch of the mesonephric artery which runs anteriorly along the lateral edge of the gland. This artery runs directly from the mesonephric artery to the gland. The corresponding artery to the right gland remains (fig. 8). Aside from this modification there is no further change in the external arrangement of the arteries. Within the glands there is a slight increase in the number of capillaries and the nature of their walls undergoes a marked change. When first formed, the walls of the capillaries contain no connective tissue, but as the period of incubation draws to a close, a small amount of it may be seen in the walls of the larger ones. If any connective tissue is present in the walls of the smaller branches, the amount is so small that it cannot be seen in sections prepared by any of the ordinary methods.

The glands of the adult bird

In the adult bird, the adrenal glands lie just anterior to the bifurcation of the postcava, one on each side of the median line. They are about 1.5 cm. long and 0.5 cm. wide at the widest part. The right gland is roughly triangular, while the left is oval in outline (fig. 8, *ad.*). The internal arrangement of the cortical and chromaffin substances shows no change from the condition found in the embryo at the close of the period of incubation. In the natural increase in size of the glands it is the cortical substance, chiefly, which has increased in mass so that there is much more of this in proportion to the chromaffin substance than was present in the embryo. The same relation between the blood vessels and the tissue of the gland is found here as was described in the well advanced embryos. The sinusoids pass between the groups of chromaffin cells, while the capillaries lie in the cortical substance but each encroaches to a certain extent upon the territory of the other.

The trunks of the venous trees have increased greatly in size and form large central vessels which may be compared to the central vein of the adrenal gland of mammals. The sinusoidal character of the venous blood vessels persists in the adult and even in the largest vessels very little connective tissue is found in the walls. Near the close of the period of incubation, the venous blood was found entering the postcava by means of two separate veins from each gland. This condition is not found in the adult. The two veins anastomose on the ventral surface of the gland and the blood from both venous trees enters the postcava through a common vein (fig. 8).

The arterial system of the glands is essentially the same as that described for the embryo. Since this is true, it is evident that the mesonephric arteries from which they derived part of their blood supply have persisted as the renal arteries.

In the adult, the blood enters the gland by means of several arteries (fig. 8). It may enter the left gland directly from the aorta or through the anterior or posterior branches of that artery which arises from the renal artery and runs along the lateral border of the gland. The supply of the right gland differs from that of the left in that there is no direct connection with the aorta and in that there is a direct connection with the renal artery. The blood leaves both glands in the same way, entering the postcava at the bifurcation by means of a single vein from each gland.

SUMMARY

1. The anlagen which give rise to the cortical substance of the adrenal glands of birds appear as groups of cells which migrate dorsally from the peritoneal epithelium.

2. The chromaffin substance is derived from indifferent cells which wander in from the anlagen of the prevertebral sympathetic plexuses.

3. The chromaffin substance of the glands lies in contact with the venous blood vessels. The vessels of the arterial system are found almost entirely in the cortical substance. In general, this is the same condition as was found by Flint in the adrenal glands of mammals.

4. The entire venous system is derived from the subcardinal veins. Within the glands, the vessels of this system are sinusoidal in character.

5. During the period that there is the greatest influx of cells from the anlagen of the prevertebral sympathetic plexuses there is also the greatest activity in the development of the vascular systems. Is it possible that the relationship of cause and effect exists between the simultaneous activity in the development of these distinct systems of the adrenal glands.

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